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Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (*Salmo salar*)

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ABSTRACT

A feeding trial was conducted to test the growth potential, nutritional utilization, liver health and fillet sensory parameters of sea-water Atlantic salmon (*Salmo salar* L.) fed diets with increasing substitution of fish meal with insect meal. The insect meal was produced from black soldier fly larvae (*Hermetia illucens*, L.). Triplicate sea-cages of salmon were fed one of four isonitrogenous and isolipidic diets for 16 weeks. The control diet (IM_0) contained 100 g kg^{-1} fish meal, which was replaced up to 100% with insect meal (33% (IM_{33}), 66% (IM_{66}) and 100% (IM_{100})), corresponding to dietary insect meal inclusion levels at 50 g kg^{-1}, 100 g kg^{-1} and 150 g kg^{-1}, respectively. Replacing the dietary fish meal with insect meal did not affect the apparent digestibility coefficients (ADC) of protein, lipid, amino acids and fatty acids, or the digestive enzyme activities. Feed intake, daily growth increase, and feed conversion ratio were also unaffected by the inclusion of insect meal in the diets. Whole body protein, lipid and amino acid composition were not affected by dietary substitution of fish meal with insect meal, while the whole body fatty acid composition generally reflected that of the diets. Liver lipid accumulation was not affected by replacing the fishmeal with insect meal, as assessed by both histological examinations and chemical analyses. The sensory testing of the fillet revealed only small changes in the fillet sensory quality. In general, this study showed that a total replacement of fish meal with black soldier fly larvae meal in the diets of sea-water Atlantic salmon was possible without negative effects on growth performance, feed utilization, nutrient digestibility, liver traits or the sensory qualities of the fillet.

1. Introduction

The choice of ingredients and formulation of the fish diets can greatly influence the environmental impact of the aquaculture industry (Boyd and McNevin, 2015). Therefore, continuous improvement in this sector is crucial. Finding nutritionally appropriate and sustainable alternatives to fishmeal (FM) and –oil is an area of intense research, with possible alternative sources of ingredients coming from terrestrial plants, animal by-products, microalgae, macroalgae or insects, to mention some (Barroso et al., 2014; Boyd and McNevin, 2015; Gatlin et al., 2007; Olsen and Hasan, 2012; Wan et al., 2018). The interest in insects as feed ingredients for terrestrial and aquatic animals continues to grow every year, with increasing numbers of new scientific articles being published on the subject (Vargas-Abúndez et al., 2018; Barroso et al., 2014; Belghit et al., 2018a; Borgogno et al., 2017; Dumas et al., 2018; Lock et al., 2016; Magalhães et al., 2017; Van Huis, 2013; Veldkamp et al., 2012, Nogales-Mérida et al., 2018). The black soldier fly (BSF) (*Hermetia illucens*) larvae is considered an important candidate species to be used for animal feeds (Cammack and Tomberlin, 2017; Van Huis, 2013). Since the 1970s, this species has been used as a protein source in animal feed, mainly due to its ability to convert food waste (vegetable, fruit, factory waste, and animal tissues) into high-quality protein (Hale, 1973; Newton et al., 1977). The research and industrial-scale production of BSF larvae as feed ingredients have been intensified the last few years (FAO, 2013; Wang and Shelomi, 2017).

Available documentation of the nutritional composition and value of different insect species considered as candidates for use in animal feeds has become substantial (Alegbeleye et al., 2012; Barroso et al., 2014; Henry et al., 2015; Makkar et al., 2014). BSF larvae contain high amounts of protein (≈ 40% of dry weight (DW)) and have a well-balanced profile of essential amino acids (AA) (Henry et al., 2015; Liland et al., 2017; Wang and Shelomi, 2017). The larvae of BSF are also a

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good source of lipids, reaching up to 30% lipids (on DW basis) if grown on optimal growth media. The lipid is dominated by saturated fatty acids (FA), being especially rich in the medium-chained FA 12:0 lauric acid (Liland et al., 2017; Sealey et al., 2011; St-Hilaire et al., 2007). Replacement of FM with BSF larvae meal in aquafeeds without negative effects on growth or performance has successfully been demonstrated in some fish feeding trials (Belghit et al., 2018a; Cummins et al., 2017; Dumas et al., 2018; Elia et al., 2018; Lock et al., 2016; Magalhães et al., 2017; Renna et al., 2017), but not in others (Gasco et al., 2016; Kroeckel et al., 2012; St-Hilaire et al., 2007). Replacement of dietary FM with BSF meal reduced the growth of juvenile turbot (165–756 g insect meal (IM) kg^{-1} diet, *Psetta maxima*) and rainbow trout (300 g IM kg^{-1} diet, *Oncorhynchus mykiss*) (Kroeckel et al., 2012; St-Hilaire et al., 2007). We previously demonstrated that it is possible to include as much as 600 g kg^{-1} of IM in combination with insect oil in the diets of fresh-water Atlantic salmon without affecting the growth performance or the feed utilization (Belghit et al., 2018a). In the current trial, we aimed to study the effects of partial or total dietary replacement of FM with IM on Atlantic salmon in the sea-water stage. By rearing the fish up to a typical commercial slaughter size (~4 kg) we could gain consumer-relevant knowledge on how such a dietary change would affect both the nutritional and sensory quality of the fish fillet. To our knowledge, this is the first trial using dietary insect ingredients to grow Atlantic salmon up to slaughter-size.

2. Materials and methods

2.1. Experimental diets and feeding trial

2.1.1. Diets

The IM used in this study was produced from BSF larvae by Protix Biosystems BV (Dongen, The Netherlands). The larvae were grown on media partially containing seaweed (ground seaweed (*Ascophyllum nodosum*) mixed with organic plant-derived waste (60:40)). At the end of an eight-day growth period, the larvae were mechanically separated from the feeding media, washed and partially defatted before being dried and ground to make the IM. The nutritional composition of the IM is given in the supplementary Table 1. The diets were produced by Cargill (Dirdal, Norway), and supplemented with 1% yttrium oxide as an inert digestibility marker. Four experimental diets were formulated to be isonitrogenous (39% crude protein), isolipidic (29% crude lipid) and isoenergetic (25 MJ/kg DM gross energy) (Table 1). The control diet (IM_0) contained the protein sources FM and plant-based protein (20:80, *w*/w). The main lipid sources in all the diets were fish oil and vegetable oil (33:66, w/w). Three experimental diets were formulated, in which 33% (IM_{33}), 66% (IM_{66}) and 100% (IM_{100}) of the FM was replaced with IM, corresponding to dietary IM inclusion levels of 50 g kg^{-1}, 100 g kg^{-1} and 150 g kg^{-1}, respectively (Table 1). The diets were balanced to provide for the requirements of essential AA (methionine and lysine were added). Additional fish oil was included in the diets with less fishmeal (IM_{33}, IM_{66} and IM_{100}) to ensure sufficient dietary long-chained highly unsaturated FAs (LC-HUFAs).

2.1.2. Feeding trial and facilities

The feeding trial was conducted at Gildeskål Research Station (GIFAS) in Langholmen, Inndyr, Norway (67°N, Northern Norway) during August–December 2017, following the institutional and national guidelines for the care and use of animal, and approved by the National Animal Research Authority in Norway. Post-smolt Atlantic salmon were randomly distributed among 12 sea-cages (n = 3) (5x5x5m; 125 m^3; 90 fish per cage), with a water temperature ranging between 7 °C (December) and 13 °C (August). Prior to the start of the feeding trial, fish were acclimated to the environmental conditions for two weeks. The fish were fed one of the four diets (Table 1) during 114 days. Each diet was distributed by hand until visual satiation. Two daily meals (or 1 meal, due to the light conditions) were provided with a minimum of four hours between the meals. Uneaten feed was collected and pellets weighed and subtracted from the total daily feeding.

Table 1
Formulation, proximate composition and amino acid composition (all analyses on wet-weight basis) of the four experimental diets fed to Atlantic salmon (*Salmo salar*).

	IM_0	IM_{33}	IM_{66}	IM_{100}
Ingredients (%)				
Fishmeal LT94	10	6.67	3.33	0.0
Insect meal	0.0	4.91	9.84	14.75
Soy protein concentrate	25	25	25	25
Corn gluten meal	7.5	7.5	7.5	7.5
Wheat gluten meal	3.35	4.51	5.7	6.88
Pea protein concentrate 55	8.8	6.8	4.8	2.84
Fish oil	10.18	11.70	13.23	14.76
Rapeseed oil	20.95	18.86	16.79	14.73
Binder	12.32	12.08	11.72	11.24
Additives	1.89	1.96	2.1	2.29
Yttrium	1.0	1.0	1.0	1.0
Proximate analysis				
DM (%)	93	93	94	95
Crude Protein (%)	38	38	39	39
Crude Lipid (%)	29	29	29	29
Ash (%)	4.6	4.6	4.5	4.5
Carbohydrates (%)	11.6	11.5	11.5	11.4
Gross energy (MJ/kg)	24.6	24.9	24.8	25.0
TBARS (nmol/g)	3.0	3.4	4.2	4.9
Amino acid composition (g kg^{-1} diet)				
Essential amino acids				
His	8.5	8.6	8.0	8.8
Ile	14.0	15.0	14.0	15.0
Leu	33.5	34.0	32.7	34.0
Lys	20.5	20.0	19.5	20.0
Met	10.0	10.0	10.0	10.0
Phe	20.0	20.5	19.0	20.0
Thr	14.5	14.5	14.0	14.0
Val	16.5	17.5	16.5	18.0
Arg	22.5	22.0	20.0	20.6
Non-essential amino acids				
Ala	19.0	19.5	19.0	19.7
Asp	36.0	35.6	34.6	34.6
Glu	73.0	75.0	75.0	79.0
Gly	16.5	16.0	15.0	15.6
Hyp	1.0	0.8	0.5	0.3
Pro	23.5	25.0	25.0	27.5
Ser	20.0	20.0	19.5	20.3
Tau	0.5	0.3	0.2	0.0
Tyr	13.5	14.5	14.5	16.0

IM_0 = diet without insect meal (IM) inclusion; IM_{33}, IM_{66} and IM_{100} = 33, 66 and 100% replacement level of FM with IM, respectively. DM = dry matter; TBARS = Thiobarbituric acid-reactive substances.

2.2. Sampling

Fish were sampled at the start of the trial and at the end of the trial (day 114). At all samplings the fish were anaesthetized with Tricaine methane-sulfonate (MS-222), individually weighed and body length measured. The fish were examined externally to check for possible abnormalities. Liver and viscera were removed and weighed for calculation of organosomatic indices. At the final sampling (day 114), faeces were collected by manual stripping from 30 fish per sea-cage, pooled per sea-cage and frozen on dry ice for digestibility measurements (n = 3). From additional 6 fish per tank, the whole digestive tract was dissected, cleaned of attached adipose tissue and divided into proximal (PI), mid (MI) and distal (DI) intestine. Digesta from the PI1 (the proximal half of PI), PI2 (the distal half of PI), MI, DI1 (the proximal half of DI) and DI2 (the distal half of DI) of fish from the same tank was pooled and snap-frozen in liquid N_2 for the analysis of trypsin activity and total bile acids level (n = 3). The empty intestinal segments (PI, MI, and DI) were frozen for the brush border enzyme activity

analysis (n = 18). Blood was collected from the caudal vein by means of heparinized medical syringes from six fish per sea-cage and haemoglobin was measured in each individual sample (n = 18). Plasma was separated from the red blood cells by centrifugation (3000 g for 15 min at 4 °C), pooled per sea-cage and frozen in liquid N_2 (n = 3). For analysis of proximate composition, six whole fish per cage were pooled, homogenized and samples frozen on dry ice (n = 3). Four salmon from each sea-cage were gutted, dissected and filleted at GIFAS; the fillets were vacuum-packaged in plastic bags, packed with wet ice in polystyrene boxes and shipped over-night to NOFIMA (Ås, Norway) for sensory testing. Individual liver samples were taken from six fish per cage (only the fish fed the IM_0 and IM_{100} diets) for histological assessment of lipid droplets as well as for lipid class analyses (n = 18). For histological analyses, samples of ~0.5 × 0-5 × 1.0 cm were cut from the midsection of each liver, put in a tissue processing/embedding cassette (Simport, Quebec, Canada) and fixed in 4% formaldehyde in 1xPBS for 24 h. The samples were then infiltrated with sucrose in increasing concentrations (10%, 20% and 30% w/v sucrose in 1xPBS solution, ~24 h in each solution). Tissue was cut out from the lower-mid section of the liver for lipid class analysis and flash-frozen in liquid N_2. All frozen samples were stored at −80 °C.

2.3. Analysis of chemical composition

Total nitrogen was analyzed on freeze-dried, ground samples (feed, whole fish and faeces) using a CHNS elemental analyser (Vario Macro Cube, Elementar Analysensysteme GmbH, Langenselbold, Germany) and quantified according to Dumas (1831). The instrument was calibrated with ethylene diamine tetra acetic acid (EDTA) (Leco Corporation, Saint Joseph, MI, USA). Sulfanilamide (Alfa Aesar GmbH & Co, Karlsruhe, Germany) and a standard meat reference material (SMRD 2000, LGC Standards, Teddington, UK) was used as the control sample.

Analysis of amino acids (not including cysteine and tryptophan) of the feed, whole fish and faeces was carried out by ultra-performance liquid chromatography (UPLC, Waters Acquity UPLC system) coupled with a UV detector (Espe et al., 2014). Wet, ground samples equivalent of 30–40 mg of protein were hydrolysed in 6 M HCl at 110 °C, the residue was diluted in MilliQ-Plus water and filtered through a syringe-driven filter. Prior to the instrumental analysis, a derivatisation agent (AccQ.Tag™, Waters, Milford, MA, USA) was added to each sample. Finally, amino acids were separated by UPLC (column: Aquity UPLC BEH C18 1.7 μM, Waters, flowrate 0.7 ml min^{-1}) and results integrated by Empower 3 (Waters). Amino acids were quantified using standards from Sigma (St. Louis, MO, USA).

Starch in the feeds was quantified using an enzymatic method according to Hemre et al. (1989). Starch in 0.5 g freeze-dried, ground material was hydrolyzed with the heat-stable enzymes amylase (Termamyl-120 L; Novo-Industries, Bagsværd, Denmark) for 30 min at 80 °C and amyloglucosidase (EC 3.2.1.3.; Boehringer, Ingelheim, Germany) for 30 min at 60 °C. Glucose was subsequently measured spectrophotometrically as nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) at 340 nm after a hexokinase/glucose-6-phosphate dehydrogenase reaction using a Maxmat PL multianalyser (Montpellier, France). Starch concentration was calculated as the difference in glucose concentration before and after enzymatic breakdown. Dextrin was used as reference material.

Neutral lipid (storage fat) content of the whole fish homogenates was determined gravimetrically after ethyl-acetate extraction, while the fat in feed and faeces after acid hydrolysis and extraction with diethyl ether. Energy density (kJ g^{-1}, on wet-weight basis) was calculated by assuming caloric values of 39.7 J mg^{-1} for lipids, 18.2 J mg^{-1} for starch and 17.1 J mg^{-1} for proteins.

FA analysis was performed on feed, whole homogenized fish and faeces by gas-chromatography (GC) as previously described by Jordal et al. (2007), modified after Lie and Lambertsen (1991). Briefly, lipids from the samples were extracted using chloroform/methanol (2:1, v/v). The extracted lipids were filtered and the remaining samples were saponified and methylated using 12% BF3 in methanol. The FAs were detected by a flame ionization detector (FID) and identified by retention time using a standard mixture of methyl-esters (Nu-Chek Prep, Elyain, MN, USA) to determine the FA composition (area %). All samples were integrated using the software Chromeleon® version 7 (Thermo Scientific, Waltham, MA, USA). Amount of FA per gram sample was calculated using 19:0 methylester as an internal standard.

Yttrium oxide concentrations in freeze-dried feed and faeces was analyzed according to Otterå et al. (2003). Briefly, yttrium oxide was quantified by ICP-MS after wet digestion in a microwave oven (Otterå et al., 2003).

Thiobarbituric acid-reactive substances (TBARS) were determined in the feed by a method modified from Schmedes and Hølmer (1989). Homogenized samples (0.2 g) were weighed into screw-capped glass tubes and added 4.0 ml of chloroform: methanol (2:1, v:v) and 0.2 ml butylated hydroxytoluene. Samples were purged with N_2, and tubes were closed and incubated with constant shaking for 30 min at room temperature. Thereafter, 2.0 ml of a saturated EDTA solution was added and the tubes were centrifuged for 20 min at 1500 x g. A 2.0 ml aliquot of the methanol:water layer was transferred to clean screw-capped glass tubes, mixed with 2.0 ml TBA reagent (1% thiobarbituric acid in 5% trichloroacetic acid) and heated for 30 min at 100 °C. Absorption was measured at 532 nm and TBARS quantified by reference to an external standard (Malondialdehyde (MDA)).

2.4. Digestive enzyme activity and total bile acids level determination

Freeze-dried digesta from PI1, PI2, MI, DI1 and DI2 was mixed thoroughly with cold distilled H_2O (1:10, w/v) on a rotating shaker at 4 °C for 10 min. After centrifugation (13,000 g, 4 °C, 10 min), the supernatants were collected into 2 ml Eppendorf tubes, frozen in liquid N_2 and stored at −80 °C. Prior to fast freezing, supernatants for total bile acids determination were subjected to sonication for 60 s at 4 °C. Trypsin activity was measured using benzoyl arginine p-nitroanilide (Sigma no. B-4875, Sigma Chemical Co., St. Louis, MO, USA) as substrate modified from (Kakade et al., 1973). As bovine trypsin shows a very different activity than that of salmon, the standard curve was not used for the calculation but to check if the assay worked. The trypsin activity is expressed as the difference in absorbance between the test and blank tube per mg dry matter (ΔOD/mg dry matter). Total bile acids were determined using the Enzabile test kit (catalog no. 550101, BioStat Diagnostic Systems, Cheshire, U.K.) and a curve derived from standardized taurocholic acid solution.

The PI, MI, and DI tissue was homogenized in cold tris-mannitol buffer (1:20 w/v) containing the serine protease inhibitor (24 μg/ml), 4-(2-aminoethyl)benzenesulfonyl fluoride HCl (Pefabloc® SC; Pentapharm Limited, Basel, Switzerland), using an Ultra Turrax® homogenizer (IKA, Staufen, Germany) followed by sonication at 4 °C for 15 s. The homogenates were frozen in liquid N_2 in aliquots and stored at −80 °C awaiting analysis. The leucine aminopeptidase (LAP) activity was determined using L-leucine-β-naphthylamide as substrate (Krogdahl et al., 2003). The enzyme activity is expressed as specific activity, normalized by the tissue protein. The protein concentration of homogenates was determined using the BioRad® Protein Assay kit based on the Bradford dye-binding method (BioRad Laboratories, Munich, Germany).

2.5. Haemoglobin and plasma metabolite assays

Haemoglobin was measured using a Cell-Dyn 400 (Sequoia-Turner, Santa Clara, CA, USA) according to the manufacturer's instructions, using Para 12 control blood (Streck) for calibration. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose (Glu), free fatty acids (FFA), total protein (Tprot) and sodium (Na^+)

were analyzed using a clinical bioanalyser (Maxmat PL analyser, Montpellier, France) and controls to determine the fraction cholesterol and triacylglycerol (TAG) concentrations.

2.6. Histology

2.6.1. Preparation of histological slides

The fixed liver samples were washed in PELCO® Cryo-Embedding Compound (Ted Pella, Altadena, USA) before being frozen in 22x22x20 mm Peel-a-way embedding molds (Ted Pella, Altadena, CA, USA) using the same medium. An ice bath made of dry ice mixed with 70% ethanol was used for the initial freezing. The frozen blocks were then put in a freezer at −22 °C for a minimum of 24 h and cut in 10 µm slices with a Cryostat Leica CM19500 (Leica, Wetzlar, Germany) set at −25 °C. The slides were then air-dried at room temperature in a protected environment for 24 h before being stained with Oil red O (Sigma-Aldrich) and hematoxylin (Sigma-Aldrich).

2.6.2. Quantification of lipid droplets

Visiopharm Integrator System (Version 3.6.5.0, Visiopharm, Haersholm, Denmark) was used to randomly select 20 frames covering the area of interest. An Axioskop microscope (Zeiss, Oberkochen, Germany) equipped with an Olympus DP72 camera (Olympus, Tokyo, Japan) was used for the microscopy. The pictures were taken at 20× magnification and processed with the java-based image processing and analysis tool ImageJ (Version: 1.50i). By actively adjusting the threshold colors on a red-, green-, blue (RGB) scale, one could select a certain range of hues, which in this case were the lipid drops colored red (Fig. 1). The “analyze particles”-function was used to obtain data on the size and number of lipid droplets as well as the percentage of the area of interest covered by lipids. Settings were adjusted to only count particles with a minimum of 15 pixels to avoid irrelevant/non-lipid selections.

2.7. Lipid class analyses

Liver lipid was extracted in chloroform/methanol 2:1 (Merck, Darmstadt, Germany) with 1% BHT (2,6-di-tert-butyl-4-methylphenol; Sigma–Aldrich). The samples were analyzed for relative and absolute amounts of lipid classes by high performance thin layer chromatography (HPTLC) as previously described by Torstensen and Frøyland (2004).

2.8. Sensory testing

The center part of the fillet was divided into 3 × 3 cm skin- and boneless sections that were vacuum-packed and frozen at −40 °C for two months. Thawing was performed at 0–1 °C before sample preparation the next day. Fillet sections from salmon from each sea-cage were pooled and evaluated as raw and baked in triplicate by each assessor (n = 3). Baking was performed in a combi-oven (Electrolux Air-o-steam, Model AOS061EANQ) at 75 °C (50% steam/50% heat) until a core temperature of 59 °C was reached. Samples were cooled at 0–1 °C.

The sensory panel consisted of ten trained assessors with an average of 15 years of experience in sensory analysis (see Ådland Hansen et al. (2012) for details regarding the sensory panel and laboratory design). The raw and heated samples were served in white plastic containers with a lid at a temperature of 20 °C. The panelists recorded their results on a 15-cm non-structured continuous scale with the left side of the scale corresponding to the lowest intensity, and the right side corresponding to the highest intensity. The responses were transformed into numbers between 1 (low intensity) and 9 (high intensity). In a pre-test session, the assessors were calibrated on samples that were considered the most different (IM_0 versus IM_{100}) on the selected sensory attributes as shown in the supplementary Table 2. Tap water and unsalted crackers were available for palate cleansing.

2.9. Calculations

Growth and nutritional indices were calculated as followed:

Condition factor (CF) = body weight (g)/Length^3 (cm)*100.
Daily growth index (DGI) = 100 x ((final body weight) $^{1/3}$ - (initial body weight)$^{1/3}$) day^{-1}.
Hepatic Somatic Index (HSI) = liver weight (g)/body weight (g) * 100.
Visceral Somatic Index (VSI) = viscera weight (g)/body weight (g) * 100.
Feed conversion ratio (FCR) = feed intake (g)/fish weight gain (g).
Protein production value (PPV) = (final protein content – initial protein content)*protein fed $^{-1}$.
Lipid production value (LPV) = (final lipid content – initial lipid content)*lipid fed $^{-1}$.
Apparent digestibility (AD) = $100 - (Y_d{*}CX_f){*}(Y_f{*}CX_d)^{-1}{*}100$, where d is diet, f is faeces, Y is yttrium concentration, and CX is nutrient concentration.

Fatty acid productive value (FAPV):

$$FAPV = \frac{\text{g FA per tank at end of trial–g FA per tank at start of trial}}{\text{g FA eaten in total per tank during 16 week feeding trial}}$$

2.10. Statistical analysis

All statistical analyses were performed using the free software environment R (R Development Core Team, 2011). All data except for results from analyses of lipid droplet size were statistically evaluated by a regression design using a linear model (lm) and one-way ANOVA (Tukey test) to find differences due to dietary treatments. For data from the histological assessment of liver area covered by lipid (random factors: tank and picture). For lipid droplet size, generalized linear models (*glmer*) were used due to the gamma distribution of the data (random factors: tank and picture). All data were analyzed for homogeneity of variance using a Levene's test and for normality using a Shapiro Wilk's

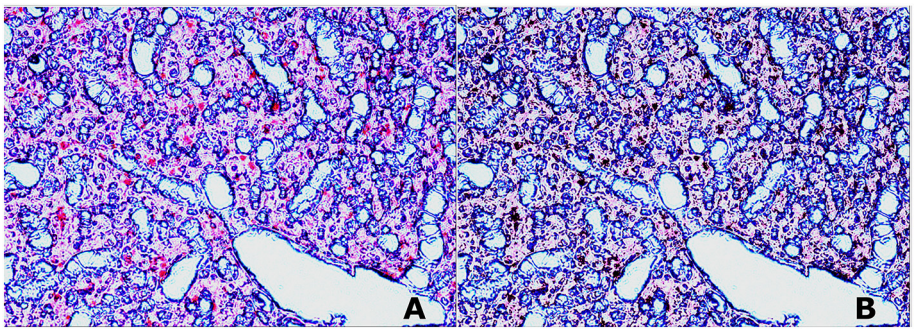

Fig. 1. The storage of neutral lipids in the cryo-cut liver tissue was stained red with Oil Red O (A) and image analyses tools were used to assess size and number of lipid droplets per cell. Hepatocytes selected by grid selection for lipid measurements were manually color adjusted to select the dyed lipid (selected area marked in black in B) and compared to the original image (A). The size of each individual lipid droplet was then measured in pixels using automatic quantification tools. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

test, as well as being evaluated graphically by QQ-plots before utilizing parametric tests. Differences were regarded as significant when $P \leq 0.05$. All data are presented as means and pooled standard error (SE), if not otherwise stated.

3. Results

3.1. Dietary composition

Analyzed proximate compositions of the experimental diets were similar to calculated compositions. All diets were similar in dry matter, protein, fat, energy and carbohydrates (Table 1). The level of peroxidation product (TBARS) increased slightly with increasing replacement of dietary FM with IM (Table 1). The diets had close to identical concentrations of the essential amino acids (Table 1). Dietary replacement of FM with IM resulted in lower concentrations of the non-essential AAs hydroxoproline and taurine, while leading to higher tyrosine, proline and valine concentrations (Table 1). Surprisingly, the concentration of the essential AA was lower in the diet where FM was replaced with IM at 66% than IM_{33} and IM_{100} diets (Table 1). This difference in AA composition might be due to the various protein sources included at different levels in the three experimental diets (IM_{33}, IM_{66} and IM_{100}) (Table1).

An increased dietary IM inclusion also led to some changes in dietary fatty acid composition (Table 2), such as an increase in the content of the medium-chain fatty acid 12:0 (lauric acid). The IM-containing diets were also slightly higher in the LC-HUFAs eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). An increase in the total n-3 fatty acids combined with a reduced n-6 fatty acid content led to an increased dietary n-3/n-6 ratio.

3.2. Growth performance and somatic index

The fish almost tripled their weight during the 114 day feeding trial (initial weight: 1398 g, final weight: 3686 g) ($n = 3$). There were no significant effects of IM inclusion on final weight, weight gain or any of the growth or feed intake parameters (DGI, SGR, HSI, VSI, FI, FCR, CF, PPV and LPV) (Table 3).

Table 2
Fatty acid composition (g/100 g) and total fatty acids (FA, mg/g of wet weight) of the four experimental diets fed to Atlantic salmon.

	IM_0	IM_{33}	IM_{66}	IM_{100}
12:0	< LOQ	0.5	1.2	2.3
14:0	2.2	2.7	3.2	3.6
16:0	8.5	8.8	9.0	9.0
18:0	3.0	3.0	3.0	3.0
18:1n-9	40.0	36.0	33.0	30.0
18:1n-7	2.5	2.3	2.2	2.0
18:2n-6	14.0	13.0	12.0	11.0
18:3n-3	6.5	5.7	5.6	5.0
18:4n-3	1.4	1.6	2.0	2.0
20:4n-6 ARA	0.2	0.2	0.2	0.3
20:5n-3 EPA	3.0	3.5	4.0	4.4
22:5n-3 DPA	0.3	0.3	0.4	0.4
22:6n-3 DHA	2.9	3.4	4.0	4.0
Sum saturated FA	15.0	16.0	17.0	19.0
Sum MUFA	55.0	53.0	52.0	50.0
Sum EPA + DHA	6.0	7.0	8.0	8.5
Sum n-3	15.0	15.0	16.5	17.0
Sum n-6	14.0	13.0	12.3	11.6
Sum PUFA	29.0	28.0	29.0	29.0
n-3/n-6	1.1	1.2	1.3	1.4
Total FA (mg/g)	265	265	248	277

IM_0 = diet without insect meal (IM) inclusion; IM_{33}, IM_{66} and IM_{100} = 33, 66 and 100% replacement level of FM with IM, respectively. LOQ: limit of quantification (0.01 mg/kg sample). ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acid.

3.3. Apparent nutrient digestibility

Digestibility of crude protein, crude lipid, amino acids and fatty acids was not affected by dietary IM inclusion ($n = 3$) (Table 4).

3.4. Digestive enzyme activity and total bile acids level

No diet effect was observed for the activity of brush boarder enzyme leucine aminopeptidase ($n = 18$), nor for the trypsin activity (n = 3) or total bile acids level (n = 3) in the digesta (Table 5).

3.5. Whole fish composition

Replacing FM with IM had no significant effects on whole fish dry matter (39.4 ± 0.6), crude protein (17.1 ± 0.2), crude lipid (20.7 ± 0.8), ash (1.7 ± 0.0) (all shown as % of wet weight) or amino acid composition ($n = 3$) (Table 6). The whole body FA composition of the salmon was, however, significantly affected by IM inclusion (n = 3) (Table 7). By including IM in the diets, the concentration of lauric acid (12:0) increased (0.5–1.6% of the whole body total FA in the IM fed fish), while this FA was below the quantification limit in the whole fish fed diets without IM (IM_0) (Table 7). The concentration of EPA and DHA as well as the n-3/n-6 ratio increased significantly in a linear manner with increasing inclusion of IM at 33 (IM_{33}), 66% (IM_{66}) and 100% (IM_{100}) in the diets (Table 7). The concentrations of ARA, PUFA and stearidonic acid (18:4n-3) was not affected by dietary inclusion of IM (Table 7).

3.6. Fatty acid productive values

The fatty acid productive values (FAPV) calculated for the fish fed the different experimental diets, showed few dietary effects (supplementary Table 3) ($n = 3$). The FAPVs of the LC-HUFAs EPA and DHA decreased, however, significantly in the fish fed with increasing inclusion of dietary IM (as an example, FAPV for EPA decreased significantly from 0.44 in the IM_0 fed fish to 0.34 in the IM_{100} fed fish (supplementary Table 3).

3.7. Haemoglobin and plasma clinical chemistry

Dietary inclusion of IM did not affect the concentration of Hg ($n = 3$), ALT (n = 3), FFA ($n = 18$), TAG (n = 18), Chol (n = 3), Tprot (n = 3) or Na^+ (n = 18) in the plasma (Table 3). AST ($n = 18$) values significantly decreased due to increasing substitution of FM with IM in the diets, whereas a higher Glu (n = 18) level was found in fish fed the IM_{66} diet (Table 3).

3.8. Assessment of liver lipid storage

A total of 80′720 individual lipid droplets were measured in the liver of the IM_0 and IM_{100} fed fish by analyzing the cryo-cut liver slides (n = 18). The size of the lipid droplets ranged from the 15px cutoff in the bottom to the largest ones measuring 5863 and 6647 px in the IM_0 and IM_{100} fed fish, respectively. Most of the lipid droplets were, however, in the lower size range with 99.3% of the lipid droplets measuring < 300px and 88.2% < 50px. The median size of liver lipid droplets were 21 px for IM_0 and 22 px for IM_{100} and there was no significant difference in the size distribution of the droplets. The area of liver covered with stained neutral lipid was also measured in all photos taken of the histological slides (351 and 333 photos of IM_0 and IM_{100}, respectively). The percent of liver area covered by lipid in the IM_{100} fed fish was not affected by dietary treatment (0.63 ± 0.90% and 0.22 ± 0.48% of liver area covered by lipid in the IM_0 and IM_{100} fed

Table 3
Growth parameters and haemoglobin (Hg) and plasma clinical chemistry of Atlantic salmon fed diets with increasing replacement of fish meal with insect meal.

	Diets				Linear regression		*ANOVA*	
	IM_0	IM_{33}	IM_{66}	IM_{100}	R^2	*P*	Pooled SE	*P*
Growth parameters								
IW (g)	1398	1400	1386	1409	< 0.001	0.82	9.35	NS
FW (g)	3702	3650	3721	3668	< 0.001	0.91	36.51	NS
DGI	3.8	3.7	3.8	3.7	< 0.001	0.80	0.04	NS
SGR	0.9	0.8	0.9	0.8	< 0.001	0.80	0.008	NS
FI	1.9	1.9	1.9	1.9	< 0.001	0.55	0.01	NS
FCR	1.1	1.1	1.1	1.1	< 0.001	0.55	0.006	NS
CF	1.5	1.5	1.4	1.5	< 0.001	0.34	0.008	NS
HSI	1.1	1.1	1.1	1.1	< 0.001	0.33	0.28	NS
VSI	12.0	11.5	11.5	11.8	< 0.001	0.70	0.14	NS
PPV	0.3	0.3	0.3	0.3	< 0.001	0.67	0.003	NS
LPV	0.7	0.7	0.6	0.7	< 0.001	0.88	0.02	NS
Hg and plasma clinical chemistry								
Hg (g/100 ml)	9.1	9.7	10.0	9.0	< 0.001	0.95	0.16	NS
ALT (IU/l)	9.1	13.3	8.8	8.7	< 0.001	0.71	1.85	NS
AST (IU/l)	765	694	631	587	*0.2*	*0.05*	35.50	NS
Glu (mmol/l)	6.4^{a}	6.5^{ab}	7.4^{b}	6.1^{a}	< 0.001	0.96	0.20	*0.04*
FFA (mmol/l)	0.7	0.6	0.8	0.7	< 0.001	0.64	0.14	NS
TAG (mmol/l)	3.1	2.1	2.5	2.6	< 0.001	0.48	0.23	NS
Chol (mmol/l)	6.6	7.1	7.0	6.6	< 0.001	0.93	0.18	NS
T_{prot} (g/l)	41.4	44.8	45.6	42.6	< 0.001	0.66	0.89	NS
Na^+ (mmol/l)	178	176	177	174	0.18	0.10	1.2	NS

IM_0 = diet without insect meal (IM) inclusion; IM_{33}, IM_{66} and IM_{100} = 33, 66 and 100% replacement level of FM with IM, respectively. IW = initial weight; FW = final weight; DGI = daily growth increase (%/fish/day); SGR = specific growth rate; FI = feed intake (g/fish/day); FCR = food conversion ratio; CF = condition factor; HSI = hepatosomatic index; VSI = viscerosomatic index; PPV = protein productive value; LPV = lipid productive value; Hg = haemoglobin; ALT = alanine aminotransferase; AST = aspartate aminotransferase; Glu = glucose; FFA = free fatty acids; TAG = triacylglycerol; Chol = cholesterol; T_{prot} = total protein; Na^+ = sodium. Values are means and pooled standard error (SE). Significant differences $P \leq 0.05$; linear regression (R^2 = Adjusted R-squared) and one-way ANOVA (mean values in the same row with different superscript) were recorded among the dietary groups.

fish, respectively) (Fig. 1). There were no significant effects of the IM inclusion on liver lipid class composition (Table 8).

3.9. Sensory testing

Sensory scores for pleasant odor (fresh and sea-water, see supplementary Table 4) showed no significant difference between the dietary groups (Fig. 2) ($n = 3$). However, a 100% replacement of FM with IM resulted in a higher score for rancid odor of the baked salmon compared with the control group (IM_0) (supplementary Table 4). Rancid odor of the raw salmon and off-odor of the baked salmon showed the same tendencies. There were no significant flavor differences between the dietary groups, but the numerical scores for rancid flavor of the baked fillet increased with increasing inclusion level of dietary IM. No significant differences were recorded for the color scores of raw salmon between the dietary groups, but the color intensity of the cooked salmon was significantly lower in the IM_{66} group compared with the control group (IM_0) (supplementary Table 4). The raw salmon fed the IM_{100} were softer compared with the IM_{33}, while an opposite trend was observed for the baked salmon, where the salmon fed higher inclusion of IM tended to be harder. Higher moisture release was detected from the raw salmon fed the IM_{66} diet compared with the control group (IM_0) (supplementary Table 4). Overall regression analyses revealed no significant relationship between dietary inclusion level of insect meal and any of the sensory properties.

4. Discussion

In the current study, a partial or complete substitution of FM protein with IM in the diet did not negatively affect feed intake, growth performance or nutrient utilization of sea-water phase Atlantic salmon. These results are in agreement with previous studies on inclusion of BSF larvae ingredients in salmonid diets (BSF larvae meal included at 150–600 g kg^{-1} diet), where no differences on the growth parameters were reported (Belghit et al., 2018a; Dumas et al., 2018; Lock et al., 2016; Renna et al., 2017). Similarly, a partial or total dietary replacement of FM with yellow mealworm (*Tenebrio molitor*) or housefly maggot (*Musca domestica*) meal did not led to negative effects on the growth of blackspot sea bream (*Pagellus bogaraveo*) or barramundi (*Lates calcarifer*), respectively (Iaconisi et al., 2017; Lin and Mui, 2017). Interestingly, a total replacement of dietary FM with cricket (*Gryllus bimaculatus*) meal (at an inclusion level of 350 g kg^{-1} diet) increased body weight gain and specific growth rate of African catfish (*Clarias gariepinus*) (Taufek et al., 2016). However, reduced growth and feed utilization has been reported in juvenile turbot and rainbow trout when FM was replaced with BSF meal (Kroeckel et al., 2012; St-Hilaire et al., 2007). The authors of these papers speculate whether these negative effects on growth could be due to the presence of chitin in the BSF meal, which could affect the digestibility of the nutrients and therefore resulting in reduced fish growth. Kroeckel et al. (2012) also concluded that the diets with BSF were less palatable and that this had led to a reduced feed intake of the fish. Other feeding trials found that salmonids fed diets containing BSF larvae raised on fish offal or seaweeds grew better than fish fed dietary BSF larvae raised without marine nutrients (Sealey et al., 2011; Belghit et al., 2018a). The current feeding trial used an IM made from BSF grown on seaweeds. This means that the selection of substrate used to grow the insect is of potential importance for the success of the use of insect ingredients in aquafeeds. Overall, these varied results in growth performances between different trials might be due to differences in tolerance level of insect ingredients between different fish species, but also due to the various life stages of fish used for the trials.

In line with absence of diet effect on the proteinase activity (trypsin and leucine aminopeptidase) and total bile acids level in the digesta, no significant effects of including IM were found on the digestibility of crude protein, crude lipid, or AAs. The obtained values for AA apparent digestibility were comparable to those observed in other studies with European seabass (*Dicentrarchus labrax)*, rainbow trout and fresh-water

Table 4
Apparent digestibility coefficients (ADC %) of crude protein, crude lipid, amino acids and fatty acids in Atlantic salmon fed diets with increasing replacement of fish meal with insect meal.

	Diets				Linear regression		*ANOVA*	
	IM_0	IM_{33}	IM_{66}	IM_{100}	R^2	*P*	Pooled SE	*P*
CP	84	83	83	82	0.006	0.21	0.66	NS
CL	85	84	88	86	0.01	0.37	0.91	NS
Amino acid								
Ala	87	83	85	86	< 0.001	0.96	0.83	NS
Arg	93	90	91	91	< 0.001	0.68	0.66	NS
Asp	78	74	75	77	< 0.001	0.87	1.10	NS
Glu	90	87	90	91	< 0.001	0.58	0.81	NS
Gly	82	78	78	78	0.005	0.23	0.94	NS
His	87	83	84	84	< 0.001	0.57	0.92	NS
Hyp	67	n.c.	n.c.	n.c.	–	–	–	–
Ile	86	82	84	84	< 0.001	0.92	0.98	NS
Leu	89	85	87	88	< 0.001	0.81	0.82	NS
Lys	88	83	84	85	< 0.001	0.48	0.86	NS
Met	91	88	90	90	< 0.001	0.88	0.58	NS
Phe	90	87	88	88	< 0.001	0.85	0.74	NS
Pro	88	85	86	88	< 0.001	0.64	0.77	NS
Ser	86	82	84	85	< 0.001	0.85	0.80	NS
Tau	n.c.	n.c.	n.c.	n.c.	–	–	–	–
Thr	80	76	77	78	< 0.001	0.85	1.04	NS
Tyr	88	84	86	87	< 0.001	0.90	0.89	NS
Val	86	82	84	85	< 0.001	0.95	0.92	NS
Fatty acid								
12:0	n.c.	n.c.	n.c.	n.c.	–	–	–	–
14:0	92	85	86	85	0.11	0.16	1.39	NS
16:0	82	77	78	79	< 0.001	0.59	1.22	NS
18:1n-9	97	93	95	92	< 0.001	0.59	1.20	NS
18:1n-7	92	86	89	85	0.02	0.28	1.63	NS
18:2n-6	97	93	95	91	0.005	0.33	1.38	NS
18:3n-3	98	95	97	94	0.001	0.34	1.18	NS
20:1n-9	95	91	93	91	0.01	0.31	1.13	NS
18:4n-3	99	97	99	96	< 0.001	0.47	1.02	NS
20:4n-6 ARA	77	65	83	80	0.06	0.22	2.68	NS
22:1n-11	98	93	93	90	0.21	0.08	1.39	NS
20:5n-3 EPA	98	95	98	94	< 0.001	0.48	1.14	NS
22:5n-3 DPA	93	90	94	90	< 0.001	0.72	1.17	NS
22:6n-3 DHA	95	92	96	92	< 0.001	0.68	1.22	NS
Saturated FA	93	89	91	88	0.03	0.27	1.17	NS
Sum MUFA	97	93	94	91	0.05	0.23	1.20	NS
Sum EPA + DHA	97	94	97	93	< 0.001	0.58	1.20	NS
Sum n-3	98	94	97	93	< 0.001	0.43	1.21	NS
Sum n-6	97	92	95	91	< 0.001	0.34	1.39	NS
Sum PUFA	97	93	96	92	< 0.001	0.41	1.27	NS

IM_0 = diet without insect meal (IM) inclusion; IM_{33}, IM_{66} and IM_{100} = 33, 66 and 100% replacement level of FM with IM, respectively. n.c. = not calculated due to very low concentrations in either feed or faeces; ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acid. Values are means and pooled standard error (SE). Significant differences $P \leq .05$; linear regression (R^2 = Adjusted R-squared) and one-way ANOVA were recorded among the dietary groups.

Atlantic salmon fed dietary insect ingredients (Magalhães et al., 2017; Dumas et al., 2018; Belghit et al., 2018a). BSF larvae have a profile of both the essential and non-essential AAs close to that of FM, except for the content of the essential AAs methionine and lysine (supplementary Table 1) (Henry et al., 2015; Liland et al., 2017; Makkar et al., 2014). In the current study, these two essential amino acids were added to all diets in order to fulfil the requirement of Atlantic salmon (NCR, 2011; Espe et al., 2014). The addition of these essential AA to the diets could also be a reason why no changes in growth were seen in the current trial. Similarly, no differences were found in the growth of rainbow trout or Atlantic salmon fed with dietary IM supplemented with methionine and lysine (Belghit et al., 2018a; Dumas et al., 2018; Lock et al., 2016). On the other hand, feeding European seabass with IM (195 g kg^{-1} diet) did not lead to a negative effect on the growth performance, in spite of the low level of lysine in the insect-based diet (Magalhães et al., 2017). Thus, some fish species might tolerate higher FM replacement with insect ingredients without the need for additional essential AA, due to different AA requirements. Additionally, Magalhães et al. (2017) reported that the ADC of arginine, although not different in the diets, increased when fish were fed with dietary IM compared to seabass fed with plant ingredients and FM as protein sources. The high ADC of arginine in this study was probably due to the high bio-availability of this essential AA in BSF larvae (Magalhães et al., 2017). Our own studies confirmed the high levels of arginine in the IM (supplementary Table 1) and we also saw a high ADC of this essential AA (91%). Therefore, compared to plant protein ingredients, where arginine is a limiting AA (Andersen et al., 2013), the larvae of BSF are a valuable sources of this essential AA.

Non-essential AAs are not strictly needed in the diet because the fish can synthesize them themselves. They can, however, have beneficial effects on fish health and performance if present in the right concentrations (Wu et al., 2013). The IM used in the current trial contained low levels of the non-essential AAs hydropxoproline and taurine compared to a typical FM. In a previous study, we found that the hepatosomatic index increased in fresh-water salmon fed insect-based diets compared to fish fed with diets devoid of IM (Belghit et al., 2018a). We

Table 5
Digestive enzyme activity and total bile acids level in the intestine of Atlantic salmon fed diets with increasing replacement of fish meal with insect meal.

	Diets				Linear regression		*ANOVA*	
	IM_0	IM_{33}	IM_{66}	IM_{100}	R^2	*P*	Pooled SE	*P*
Trypsin (ΔOD/mg DM)								
PI1	353	423	470	356	< 0.001	0.89	90.5	NS
PI2	300	352	348	381	< 0.001	0.45	75.0	NS
MI	107	177	139	165	< 0.001	0.38	34.1	NS
DI1	92	109	111	125	0.023	0.29	22.1	NS
DI2	78	62	47	82	< 0.001	0.99	19.9	NS
Bile acids (μmol/g DM)								
PI1	209	234	228	217	< 0.001	0.91	35.5	NS
PI2	176	214	172	179	< 0.001	0.75	23.6	NS
MI	91	114	103	90	< 0.001	0.79	10.0	NS
DI1	38	39	46	39	< 0.001	0.78	7.2	NS
DI2	12	15	18	13	< 0.001	0.79	3.8	NS
Leucine aminopeptidase (μmol/h/mg protein)								
PI	460	407	391	375	0.098	0.17	30.3	NS
MI	180	187	205	175	< 0.001	0.94	11.4	NS
DI	368	331	399	336	< 0.001	0.89	38.6	NS

IM_0 = diet without insect meal (IM) inclusion; IM_{33}, IM_{66} and IM_{100} = 33, 66 and 100% replacement level of FM with IM, respectively. DM = dry matter; PI = proximal intestine; MI = mid intestine; DI = distal intestine. Values are means and pooled standard error (SE). Significant differences $P \leq 0.05$; linear regression (R^2 = Adjusted R-squared) and one-way ANOVA were recorded among the dietary groups.

Table 6
Whole-fish amino acid composition (mg/ g) of Atlantic salmon fed diets with increasing replacement of fish meal with insect meal.

	Diets				Linear regression		*ANOVA*	
	IM_0	IM_{33}	IM_{66}	IM_{100}	R^2	*P*	Pooled SE	*P*
Ala	9.9	9.8	10.1	10.0	0.16	0.10	0.05	NS
Arg	9.1	9.1	9.4	8.9	< 0.001	0.88	0.11	NS
Asp	16.3	16.0	16.4	16.7	0.22	0.06	0.09	NS
Glu	20.6	19.9	20.9	20.8	0.05	0.22	0.14	NS
Gly	9.8	9.7	10.4	9.4	< 0.001	0.72	0.18	NS
His	4.0	4.0	4.1	4.1	0.03	0.26	0.04	NS
Hyp	1.1	1.0	1.2	0.9	< 0.001	0.39	0.05	NS
Ile	6.7	6.7	6.8	6.9	0.11	0.14	0.04	NS
Leu	12.0	11.9	12.1	12.1	0.06	0.22	0.05	NS
Lys	14.8	14.6	14.8	15.2	0.23	0.06	0.09	NS
Met	4.8	4.8	4.9	4.8	< 0.001	0.98	0.03	NS
Phe	6.4	6.5	6.6	6.4	< 0.001	0.94	0.08	NS
Pro	6.6	6.5	6.9	6.6	< 0.001	0.76	0.07	NS
Ser	6.7	6.7	6.9	6.7	< 0.001	0.95	0.05	NS
Tau	0.8	0.8	0.9	0.8	< 0.001	0.77	0.01	NS
Thr	7.5	7.4	7.6	7.5	< 0.001	0.43	0.03	NS
Tyr	5.1	5.2	5.3	5.1	< 0.001	0.94	0.05	NS
Val	7.8	7.7	7.9	8.0	0.08	0.17	0.04	NS

IM_0 = diet without insect meal (IM) inclusion; IM_{33}, IM_{66} and IM_{100} = 33, 66 and 100% replacement level of FM with IM, respectively. Values are means and pooled standard error (SE). Significant differences $P \leq 0.05$; linear regression (R^2 = Adjusted R-squared) and one-way ANOVA were recorded among the dietary groups.

speculated that the low level of taurine in the IM might have affected the fat content of the liver, as the addition of taurine to a high-plant diet (low in taurine) had a positive effect on lipid metabolism and reduced liver lipid depositions in juvenile Atlantic salmon (Espe et al., 2012). In the current trial, however, a replacement of FM with IM did not affect the hepatosomatic index of the fish. Insect meal was included at a maximum level of 150 g kg^{-1} diet in the current trial, while in the previous study, 600 g kg^{-1} of IM in combination with insect oil were included in the diets of fresh-water stage salmon (Belghit et al., 2018a), which could explain the different results obtained between these two trials. Hydroxoproline is an abundant AA in animal tissues, predominantly found in collagens. Insect collagen does, however, not contain hydroxoproline. The work of Dumas et al. (2018) showed an increased digestibility of hydroxoproline when rainbow trout were fed dietary BSF meal and oil, compared to when fed diets devoid of insect ingredients. The authors also reported that the whole body content of this non-essential AA increased in rainbow trout fed with increasing level of BSF ingredients, even though the level of hydroxoproline was below detection limits in insect-based ingredients (Dumas et al., 2018). In the case of low dietary supply of hydroxoproline, animals can produce it by a post-translational hydroxylation of proline. Dumas et al. (2018) hypothesized that insect ingredients might contain some component able to promote the hydrolysis and absorption of proline and hydroxoproline in the small intestine.

An ~8% decrease in the whole body total FA was seen when replacing all the FM with IM. This was, however, not accompanied by a reduction in growth or performance, nor was it associated with any general reduction in the digestibility or retention of lipids (assessed by ADC and FAPVs of total lipid and individual FAs). Medium-chained FAs, like lauric acid (12:0), are known to be easily oxidized and to reduce lipid storage in both mammals and fish (Nordrum et al., 2003; St-Onge and Jones, 2002; St-Onge et al., 2008; Williams et al., 2006; Smith et al., 2005; Belghit et al., 2018b). Lauric acid (12:0) is the dominant fatty acid in BSF larvae, representing between 21% and 50% of total FAs (Liland et al., 2017; Oonincx et al., 2015), and increased when IM was included in the current experimental diets. The dietary lauric acid content was, however, quite low in the current trial, even in the diets where 100% of the FM was replaced with IM. This is due to the low FM content of the diets as well as the use of a partially defatted BSF meal (dietary lauric acid reaching a maximum of 6.5 g kg^{-1} diet, compared to 65 g kg^{-1} in Williams et al. (2006). The dietary lauric acid could therefore explain some of the reduced lipid storage in the IM fed fish, but is probably not the sole responsible for this.

The whole body content of EPA and DHA increased in the salmon fed with increasing inclusion of dietary IM. Additional fish oil was added to all the IM diets to replace the EPA and DHA removed by replacing the FM with IM. A slightly higher than estimated EPA and DHA content of the fish oil used in the diets led to a higher content of these FAs in the IM diets, finally resulting in higher whole body content of the same FAs. A higher dietary supply of long-chained n-3 FAs also likely reduced the need for biosynthesis of these FAs from the shorter n-3 FAs by the fish, reflected as reduced FAPV for many n-3 FAs in the IM fed fish.

Elevated activities of serum AST or ALT, are an indication of damage to liver cells. In the current trial, serum AST activity decreased by almost 24% in salmon fed with inclusion of IM in the diets. These results demonstrated that a partial or complete replacement of FM with IM in the diets did not lead to negative effects and suggest that dietary IM might have a protective effects in the liver of Atlantic salmon. To look for more changes in hepatic health, a histological evaluation of lipid accumulations was conducted on the two extreme dietary groups (IM_0 and IM_{100}). The size distribution of hepatic lipid droplets was not affected by dietary IM inclusion, neither were the hepatic TAG concentrations, as measured by lipid class analyses. Conversely, Li et al. (2017) reported histological changes in the liver of Jian carp fed defatted BSF larvae meal. In Li's study, the lipid content of hepatocytes decreased in fish fed with defatted BSF larvae meal compared to fish fed dietary FM (Li et al., 2017). The authors suggested that those observed effect might be related to the content of chitin and its derivatives found in the insect exoskeleton. These polymers have been shown to decrease the FA synthesis and to increase TAG hydrolysis in rat liver (Zhang et al., 2008). The difference in liver traits between Li et al. (2017) (IM inclusion at 10 g kg^{-1} diet) and the current findings (IM inclusion at 150 g kg^{-1} diet) could be due to differing content of chitin in the IMs used.

Modifications in fish feed ingredients can affect the color, flavor and

Table 7
Fatty acid (FA) composition (% of total FA) and total FAs (mg/g of wet weight) of the whole body of Atlantic salmon fed diets with increasing replacement of fish meal with insect meal.

	Diets				Linear regression		*ANOVA*	
	IM_0	IM_{33}	IM_{66}	IM_{100}	R^2	*P*	Pooled SE	*P*
12:0	< LOQ	0.5	1.0	1.6	–	–	0.17	–
14:0	2.0 [b]	2.4 [a]	2.7 [a]	3.0 [a]	0.97	*< 0.001*	0.11	*< 0.001*
16:0	9.1 [b]	9.4 [b]	9.5 [ab]	9.8 [a]	0.77	*< 0.001*	0.08	*0.002*
18:1n-9	38.3 [a]	36.5 [b]	34.1 [c]	32.6 [d]	0.86	*< 0.001*	0.02	*< 0.001*
18:1n-7	2.7 [a]	2.7 [a]	2.6 [b]	2.5 [b]	0.97	*< 0.001*	0.14	*< 0.001*
18:2n-6	13.2 [a]	12.7 [b]	12.3 [c]	11.9 [d]	0.95	*< 0.001*	0.06	*< 0.001*
18:3n-3	4.7 [a]	4.6 [a]	4.3 [b]	4.1 [b]	0.64	*< 0.001*	0.01	*0.01*
18:4n-3	0.8	0.8	0.9	0.9	0.32	*0.05*	0.01	NS
20:4n-6 ARA	0.8	0.8	0.8	0.8	0.34	*0.05*	0.02	NS
20:5n-3 EPA	2.2 [b]	2.3 [b]	2.5 [a]	2.6 [a]	0.71	*< 0.001*	0.02	*< 0.001*
22:5n-3 DPA	1.1 [b]	1.1 [b]	1.2 [a]	1.2 [a]	0.96	*< 0.001*	0.10	*< 0.001*
22:6n-3 DHA	4.2 [d]	4.5 [c]	4.8 [b]	5.2 [a]	0.95	*< 0.001*	0.37	*< 0.001*
Sum SFA	14.1 [d]	15.3 [c]	16.2 [b]	17.5 [a]	0.89	*< 0.001*	0.42	*< 0.001*
Sum MUFA	53.6 [a]	52.8 [a]	51.0 [b]	50.2 [b]	0.89	*< 0.001*	0.15	*< 0.001*
Sum EPA + DHA	6.4 [d]	6.8 [c]	7.3 [b]	7.7 [a]	0.92	*< 0.001*	0.15	*< 0.001*
Sum n-3	14.3 [b]	14.7 [b]	15.3 [a]	15.7 [a]	0.93	*< 0.001*	0.19	*< 0.001*
Sum n-6	15.6 [a]	15.1 [b]	14.4 [c]	14.0 [c]	< 0.001	0.36	0.07	*< 0.001*
Sum PUFA	30.2	30.1	30.0	30.0	0.98	*< 0.001*	0.02	NS
n-3/n-6	0.9 [b]	1.0 [b]	1.1 [a]	1.1 [a]	0.50	*0.005*	2.44	*< 0.001*
Total FA (mg/g)	206 [a]	196 [ab]	189 [b]	190 [b]	0.86	*< 0.001*	0.02	*0.02*

IM_0 = diet without insect meal (IM) inclusion; IM_{33}, IM_{66} and IM_{100} = 33, 66 and 100% replacement level of FM with IM, respectively. LOQ: limit of quantification (0.01 mg/kg sample). ARA = arachidonic acid; EPA = eicosapentaenoic acid; DPA = docosapentaenoic acid; DHA = docosahexaenoic acid; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids. Values are means and pooled standard error (SE). Significant differences $P \leq 0.05$; linear regression (R^2 = Adjusted R-squared) and one-way ANOVA (mean values in the same row with different superscript) were recorded among the dietary groups.

Table 8
Lipid class composition of liver (mg/g wet weight) of Atlantic salmon fed a diet without insect meal or a diet with 100% of the fish meal replaced with insect meal.

	Diets		Linear regression		*ANOVA*	
	IM_0	IM_{100}	R^2	*P*	Pooled SE	*P*
SM	4.2	3.7	< 0.001	0.60	0.22	NS
PC	19.7	16.4	0.06	0.07	1.10	NS
PS	2.1	2.0	< 0.001	0.67	0.10	NS
PI	3.1	4.4	0.02	0.16	0.43	NS
CL	0.8	0.8	< 0.001	0.31	0.01	NS
PE	4.3	5.0	< 0.001	0.32	0.55	NS
Total polar lipid	34.1	32.3	< 0.001	0.52	1.79	NS
DAG	0.8	0.7	*0.10*	*0.02*	0.01	NS
CHOL	3.3	3.3	< 0.001	0.84	0.10	NS
FFA	1.8	1.4	0.04	0.10	0.10	NS
TAG	29.4	22.4	< 0.001	0.37	3.22	NS
Total neutral lipid	35.3	27.8	< 0.001	0.33	3.23	NS
Total lipid	69.5	60.1	0.10	0.25	3.98	NS

IM_0 = diet without insect meal (IM) inclusion; IM_{100} = 100% replacement level of FM with IM. SM: sphingomyelin, PC: phosphatidylcholine, PS: phosphatidylserine, PI: phosphatidylinositol, CL: cardiolipin, PE: phosphatidylethanolamine, DAG: diacylglycerols, CHOL: cholesterol, FFA: free fatty acids, TAG: triacylglycerols Values are means and pooled standard error (SE). Significant differences $P \leq 0.05$; linear regression (R^2 = Adjusted R-squared) and one-way were recorded among the dietary groups.

the aroma of fish fillet (Olsson et al., 2003; Turchini et al., 2009), which might affect the perceived fillet quality and consequently the consumers' acceptance. In the present trial, rancid odor increased in the baked fillet obtained from salmon fed with increasing IM inclusion in the diet. The values for rancid odor, however, remained low for all dietary groups, and were similar to the values obtained for Atlantic salmon fed with other alternatives diets (Ådland Hansen et al., 2012; Rødbotten et al., 2009). The rancidity odor scores of cooked salmon were higher than in the raw salmon. An increase in fillet HUFA, like in the IM fed fish in the current trial, renders fish flesh more susceptible to oxidation during thermal treatment (Medina, et al., 1998). The elevated temperatures during the cooking process might have further stimulated the degradation of lipid components in the fillet. The initial quality of the feed ingredients might also influence the sensory profile. By measuring sensory attributes and physico-chemical parameters, Borgogno et al. (2017) found differences in perceived intensity of aroma, flavor and texture in the fillet of rainbow trout fed with dietary IM. The authors reported an increase in metallic flavor in the fillet of fish fed with increasing inclusion of IM in the diet compared to fish fed with FM (Borgogno et al., 2017). Other feeding trials, however, did not find any sensory differences in the fillet of fish fed with insect-based diets (Lock et al., 2016; Sealey et al., 2011). Based on the current trial and earlier published results on sensory attributes of the fillet of insect-fed fish, we can conclude that using insect ingredients in fish feeds leads to only marginal changes in fillet sensory quality.

5. Conclusion

In this study, we evaluated the effects of graded inclusion level of a partially defatted black solider fly larvae meal on growth performances, digestibility, nutrient utilization, liver health and fillet sensory qualities of Atlantic salmon of a commercially relevant size. Only minor effects were detected of replacing up to 100% of the fishmeal with the insect meal. Therefore, our conclusion is that the insect meal made from BSF is a nutritionally appropriate source of protein for sea-water stage Atlantic salmon.

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Fig. 2. Spider web diagram of the sensory evaluation of (A) raw and (B) baked fillets from IM_0, IM_{33}, IM_{66} and IM_{100} fed Atlantic salmon. A 100% substitution of fishmeal with insect meal (IM_{100}) resulted in a softer texture of the raw fillets compared with the fillets of fish fed with only 33% fishmeal replacement (IM_{33}). The rancidity odor of baked fillet from the IM_{100} group was significantly higher than in the control group without insect meal (IM_0). * Significant differences $P \leq 0.05$; (one-way ANOVA). IM_0 = diet without insect meal (IM) inclusion; IM_{33}, IM_{66} and IM_{100} = 33, 66 and 100% replacement level of FM with IM, respectively.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2018.12.032.

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